Blunt end (Thermo scientific)

- For cloning blunt-end PCR products generated by proofreading DNA polymerases, such as Pfu DNA polymerase. (If the DNA end structure of the PCR products is not specified by the supplier of the DNA polymerase, follow the Sticky-End Cloning Protocol).
- For cloning of blunt-end DNA fragments generated by restriction enzyme digestion. Gel-purify the DNA fragment prior to ligation and use in a 3:1 molar ratio with pJET1.2/blunt (see Table 1).
- 1. Set up the ligation reaction on ice:

Component	Volume
2X Reaction Buffer	10 µl
Non-purified PCR product or purified PCR product/other blunt-end DNA fragment	1 µl 0.15 pmol ends
pJET1.2/blunt Cloning Vector (50 ng/µl)	1 µl (0.05 pmol ends)
Water, nuclease-free	up to 19 µl
T4 DNA Ligase	1 µl
Total volume	20 µl

- Vortex briefly and centrifuge for 3-5 s.
- 2. Incubate the ligation mixture at room temperature (22°C) for 5 min.

  Note. For PCR products >3 kb, ligation can be prolonged to 30 min. Ligation times longer than 30 min are not recommended and may decrease cloning efficiency.
- 3. Use the ligation mixture directly for transformation.

  Note. Keep the ligation mixture at -20°C if transformation is postponed. Thaw on ice and mix carefully before transformation.